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(54) FISH PROTEIN ISOLATE

(71) We, NESTLE S.A., of La Tour-de-Pielz, Switzerland, a Swiss company, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention is concerned with a process for solubilisation and purification of protein from fish raw materials, to provide a taste- and odour-free water-soluble fish protein isolate with a high nutritional value.

Several methods have been devised for making a bland fish protein concentrate with a high nutritional value. With virtually all raw materials used for making such a concentrate removal of lipids is a necessity in order to ensure a bland product with sufficient stability. Generally, 0.5% by weight residual lipids in the concentrate calculated on dry matter basis, is considered as a maximum level. Solvent extraction is the normal way for such a nearly complete removal of lipids. Some of these methods have been technically adequate but none has been commercially successful due to the lack of functional properties of the resulting protein concentrate. Such functional properties include water-solubility, foaming ability, emulsifying ability and water binding capacity. A protein lacking such functional properties can only be used for protein enrichment, since it adds nutritional value, but is inert in all other respects. Furthermore, its use for protein enrichment is limited to certain types of foods such as bread in which water-solubility is not necessary. Among the functional properties that might be desirable for different uses water-solubility is the most important since it opens up broad areas for applications to all types of foods. Water-solubility is in addition a necessary condition for most of the other functional properties.

With this background, several methods have been suggested based on enzymatic hydrolysis as a means of imparting water-solubility to the fish proteins. Although being effective in this respect such methods are frequently difficult to control on an industrial scale due to variations both in the activity of the inherent fish tissue enzymes and in the activity of commercially available enzyme preparations. In addition they are relatively uneconomical since no method for recovery of the added enzymes have so far been devised.

A further possibility is alkali solubilisation, but this involves the risk of destruction of sulphur-containing amino acids thereby reducing the nutritional value. A further difficulty is the frequent formation of undesirable flavours and the release of already present flavour compounds during the solubilisation. This difficulty, together with the need for an efficient desalting process has up to now prevented the use of alkali solubilisation for making a water-soluble fish protein product.

Alkali solubilisation applied directly to the fish raw material, prior to the necessary solvent extraction of the lipids, has definite disadvantages in that no stable intermediate product is formed which means that the complete process has to be carried out at the same location.

An object of the present invention is to provide a simple, economical process for preparing a water-soluble protein isolate from fish.

The present invention thus provides a process for preparing a protein isolate from fish material, in which a fish material having a lipid content in dry matter not exceeding 0.5% by weight is treated with aqueous alkali at a pH of 10 to 12, at a temperature of 80 to 100°C for a period of time of

[*Price* 25p]

1 to 5 minutes, insoluble material is removed from the alkali solution, and thereafter the solution is desalted and deodorised.

The protein isolate obtained by the process of the invention is virtually taste- and odour-free, and has a high nutritional value, at least equal to casein. It is more specifically characterised by having a protein content between 90 and 98%, a lipid content below 0.5% and an ash content between 1 and 10% on dry matter basis.

The starting material for the process is preferably fish solids from which the lipids have been removed to the indicated degree, as for example by extraction with a solvent such as isopropanol. Solvent extraction provides a stable intermediate product, comprising a mixture of fish solids and residual solvents, which is chemically and bacteriologically stable over extended periods of time. This means that the removal of lipids may be carried out at sea and the subsequent processing of the intermediate product on land. Furthermore, the oil obtained directly is of higher quality than oil extracted from fish which has first been treated for example with alkali, and in addition low-molecular, nitrogen-containing substances, of poor nutritional value, are also removed with the solvent.

The fish material may be obtained, for example, from any one of the following:—

(1) degutted lean fish such as cod, haddock or hake

(2) filleting by-products from frozen fish factories such as V-cuts, which is a part of the fillets normally discarded due to the presence of small bones, and

(3) whole fish of pelagic species such as anchovetas, sardines, etc.

The actual method of preparation of the fish starting material is relatively unimportant, provided that it has no more than 0.5% by weight of lipids on a dry matter basis.

Treatment of the fish material with alkali is carried out under controlled conditions of pH, time and temperature so that destruction of the most sensitive amino acids, notably cystein, lysine and methionine is insignificant. Whilst sodium hydroxide is the preferred alkali, as it dissolves the protein rapidly, other alkalis may however be used provided that the positively-charged ions are capable of being removed during the subsequent desalting process described here. The alkali treatment is preferably carried out on a suspension containing 5 to 10% by weight of fish dry matter, and under the conditions herein defined it is possible to dissolve from 80 to 95% of the fish protein without any damage to the nutritional value of the protein. The actual degree of solubilisation will however depend on the fish raw material.

After alkali treatment the suspension may be cooled, for example to 50 to 60°C and insoluble matter, including undissolved protein and solid fractions such as bones removed, e.g. by filtration, pressing or centrifugation. The preferred method is continuous centrifugation, as this may be effected rapidly, resulting in a clear protein solution and in an undissolved bone and protein fraction rich in minerals. Alkali solubilisation as described permits removal of virtually all the bones, so that the fluoride content of the final protein product is very low. This bone protein fraction is preferably dried separately and used as a fodder.

The clarified protein solution is then desalted. Whilst theoretically this operation can be effected by ultrafiltration, it is technically impractical as presently-available membranes are sensitive to high pH values. Electrodialysis is an alternative, but has the disadvantage of giving rise to precipitation, which decreases the capacity of the cells. Ion exchange in columns is the most acceptable principle but this can also give rise to the formation of precipitates when the drop in pH is large, which inhibits the performance of the columns. It is accordingly preferred to carry out the desalting by exchange of the positively-charged ions by ammonium ions, as described and claimed in our copending application No. 13,086/72 (Serial No. 1 349 933). The ammonium ions are subsequently completely removed in the steam-stripping.

In a preferred embodiment of the ion exchange treatment, a strong cation exchange resin, regenerated by an ammonium salt, is used. It is especially favourable to use a macroreticular resin, e.g. Amberlite 200 due to its long life (Amberlite is a Trade Mark). The concentration of the regenerating salt solution may be from 0.1 to 5.0 N but the preferred concentration is 1.0 N. The temperature of the treated liquid is not critical but it is favourable to use a temperature of 50 to 80°C in order to reduce the viscosity of the protein solution in the column, as the dissolved proteins have the initial high molecular weight of the raw material resulting in a protein solution with high viscosity.

Whilst for certain applications, proteins giving high viscosities in water solutions are required, when a high viscosity is not necessary it is then desirable to reduce the molecular weight of the proteins, particularly by treatment with a proteolytic enzyme, so that the protein solution may be concentrated to a higher dry matter content prior to drying. The enzyme treatment should be mild enough not to change appreciably the properties of the protein other than the viscosity in solution. The enzyme used should preferably have good activity at alkaline pH values, especially in the range 8 to 10. The preferred enzymes are trypsin and bacterial proteases. The amount of enzyme needed is very small, for example 0.1 to 1.0 g per kg of protein dry matter treated. The treatment as stated is very mild, and should desirably be completed within 5 to 30 minutes. Thereafter the residual enzyme is inactivated by heat treatment, e.g. at 60 to 90°C for 5 minutes.

After desalting and enzyme treatment, if this latter is applied, the protein is deodorised, preferably by steam-stripping in order to remove all undesirable volatiles from the protein solution. These include, at previously stated, ammonium ions, flavour components formed or released in the alkali solubilisation step and any residual solvent from pretreatments such as lipid extraction. The conditions applied are selected having regard to these objectives. The stripping is preferably carried out in a packed column, with the steam entering at the bottom and the protein solution at the top, resulting in a countercurrent contact between the steam and protein solution. In general, from 0.1 to 1.0 kg of steam (at atmospheric pressure) is used per kg of protein solution. The steam leaving the column contains the various volatiles noted above, and may be condensed. The condensate may then be acidified to pH 4.5 and treated for recovery of solvent residues, whereas ammonia and volatile amines remain in the water phase and are discarded.

As the preferred steam-stripping treatment is very effective for the removal of volatiles from the protein solution, additional treatment with adsorbents such as active carbon or resin, or with chemical agents such as hydrogen peroxide are generally unnecessary. However, such treatments, if applied, may be carried out before or after the steam-stripping. Hydrogen peroxide is capable of oxidising minor traces of flavour compounds, and also bleaches the protein which may be advantageous when fatty fish with dark flesh is used as a raw material.

After the stripping, the protein may be dried to provide a final product with a moisture content low enough for stability. Prior concentration, as by reverse osmosis, freeze concentration or thermal evaporation is desirable to reduce the load on drying facilities. The method preferred according to the invention is evaporation, and if an enzymatic treatment has been applied, the protein solution may be concentrated to between 25 and 50% dry weight.

Drying may be carried out by a roller, spray- or foam drier or any other conventional drier. The drying is carried out preferably in a spray-drier at relatively low temperatures in order to avoid heat damage to the protein which would be detrimental to the nutritional value and functional properties.

The dried product is light-coloured and bland in flavour and odour. It has a high protein content, between 90 and 98% on dry weight. The product has a high nutritional value and is especially rich in available lysine, ranging from 7.0 to 9.0 g per 16 g N, depending on the raw material. The protein efficiency ratio (PER) is typically in the range 3.0 to 3.5, at least equal to that of casein as determined under identical conditions. The fat content is 0.05 to 0.5% on dry weight and the ash content between 1 and 10%. As the bones were removed after alkali dissolution, the fluoride content is low, below 25 ppm. The water-solubility of the final powder, expressed by the Nitrogen Solubility Index (determined according to the American Oil Chemist's Society Official Method Ba 11-65) lies in the range 80 to 100. By virtue of its properties, the protein isolate may be used in a wide range of foods, including sausages, meat replacements, soups, sauces, bouillons, cerials, ice-cream, milk, desserts and the like.

The following examples are given by way of illustration only. The percentages are by weight.

Example 1

1000 kg of whole herring are ground in a meat grinder and then mixed with 1500 kg of isopropanol. The slurry is stirred for 5 minutes at room temperature and the solids are then separated from the isopropanol in a centrifuge. The lipid extraction as described is repeated four further times. This treatment results in 400 kg of extracted fish mass containing 50% dry weight and 50% isopropanol. The solvent used for extraction is recovered by distillation and the fat fraction is separated. The 400 kg of extracted fish are mixed with 1600 kg of water and the slurry is homogenised. The slurry is then continuously mixed with sodium hydroxide to give a pH of 12 and heated to 80°C by steam injection. Immediately after alkali addition and steam injection the slurry is pumped through holding tubes with a residence time of two minutes and thereafter cooled to 50°C. The bones and other insolubles are separated on a vibrating screen and the protein solution is clarified by centrifugation.

The clear protein solution is pumped through a cation exchange resin column in which sodium ions are exchanged by ammonium ions, and 1800 kg solution containing 6.5% dry matter are collected from the column. 20 g of trypsin are added to the solution and the temperature is maintained at 40°C for 30 minutes. 1 kg of 35% hydrogen peroxide is then added and the temperature is raised to 70°C for 30 minutes in order to inactivate the enzyme and to bleach the protein. The bleached solution is pumped into a stripping column, in which each kg of protein solution is contacted countercurrent with 0.5 kg of steam at 100°C, and the stripped solution is cooled to 60°C. The resulting protein solution is bland in flavour, it is concentrated by evaporation to a dry matter content of 40%, and spray-dried to a moisture content of 5.0%. The product has a protein content of 91.8%, and ash content of 8.0%, and a lipids content of 0.2%. The fluoride and isopropanol are both below 25 ppm.

Example 2

1000 kg of cod filleting waste are ground in a meat grinder and then extracted in a screw extractor with isopropanol. The fish is fed into the lower end of the extractor and the isopropanol into the upper end, providing a countercurrent contact between the fish and the solvent. 1500 kg of isopropanol are used for the extraction of the 1000 kg of fish. The extracted fish mass, containing about 50% dry matter and 50% of isopropanol, continuously leaving the top of the screw is then mixed with water to give a slurry containing 10% dry matter. This slurry is pumped through a homogeniser, potassium hydroxide is added to pH 11.0 and it is heated to 100°C by direct steam injection. The hot alkaline slurry is passed through holding tubes with a residence time of one minute and immediately on leaving the tubes is passed to a decanter centrifuge where the bones and undissolved protein are separated.

The clear protein solution is cooled to 80°C and pumped through a cation exchange resin column in which potassium is exchanged by ammonium. Thereafter the solution is steam-stripped, concentrated in an evaporator to 20% dry matter and spray-dried.

The dried product has a blend flavour and the following composition:—

Protein	97.0 %
Ash	2.6
Lipids	0.4

WHAT WE CLAIM IS:—

1. A process for preparing a protein isolate from fish material, in which a fish material having a lipid content in dry matter not exceeding 0.5% by weight is treated with aqueous alkali at a pH of 10 to 12, at a temperature of 80 to 100°C for a period of time of 1 to 5 minutes, insoluble material is removed from the alkali solution, and thereafter the solution is desalted and deodorised.

2. A process according to claim 1 in which prior to removal of insoluble material the alkali solution is cooled to a temperature of 50 to 60°.

3. A process according to claim 1 or claim 2 in which the alkali solution is desalted by ion exchange whereby the cations present are exchanged for ammonium ions which are then removed from the solution by vaporisation as ammonia.

4. A process according to claim 3 in which the ion exchange is effected at a temperature of 50 to 80°C.

5. A process according to any one of the preceding claims in which after desalting the protein solution is subjected to mild enzyme treatment.

6. A process according to any one of the preceding claims in which deodorisation is effected by steam stripping.

7. A process according to any one of the preceding claims in which after deodorisation the protein solution is dried.

8. A process for preparing a protein isolate from fish material substantially as herein described with reference to Example 1 or Example 2.

9. A fish protein isolate prepared by a process according to any one of the preceding claims.

10. A food product comprising a fish protein isolate as claimed in claim 9.

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